SHORT REPORT

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Phenolic Bisabolanes from the Marine-Derived Fungus *Aspergillus* sp. MEA11

Xin Liu [1#], Wenzhen Lin [3,4,#], Ruzhen Liu [2,3,4], Minghuang Ling [3], Chiming Guo [4], Hongyan Meng [4], Ying Guo [4], Wei Xu [1,3] and Xiaona Du [2,*]

[1]*School of Life Sciences, Anhui Agricultural University, Hefei 230036, China*
[2]*Sanquan College of Xinxiang Medical University, Xinxiang, Henan 453000, China*
[3]*Key Laboratory of Marine Biogenetic Resources, Third Institute of Oceanography, Ministry of Natural Resources, 178 Daxue Road, Xiamen 361005, China*
[4]*Fujian Key Laboratory of Subtropical Plant Physiology and Biochemistry, Fujian Institute of Subtropical Botany, Xiamen 361006, China*

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Abstract: The deep sea sediment-derived fungus *Aspergillus* sp. MEA11 was examined for secondary metabolites. Chromatographic separations resulted in the identification of a new phenolic bisabolane (**1**) and seven known analogs (**3**–**7** and **8a**, **8b**). The structures were determined by ^{1}H, ^{13}C NMR, and MS data. The known compounds were identified to be 11,12-dihydroxysydonic acid (**2**), hydroxysydonic acid (**3**), aspergoterpenin B (**4**), engyodontiumone J (**5**), sydowic acid (**6**), penicipyran A (**7**), 1-hydroxyboivinianic acid (**8**). The NMR data of **7** in methanol-d_4 were reported for the first time. Compounds **6–8** exhibited inhibitory effect against α-glucosidase with IC_{50} values of 176, 89, 232 μM, respectively, which were more active than the positive control acabose.

Keywords: Phenolic bisabolanes; *Aspergillus* sp.

1. Plant Source

The fungal strain MEA11 (T11-MEA-81) was isolated from the sediments that were collected from the Atlantic Ocean (DY-26III-SMAR-S029-TVG11) at a depth of –2807 m. The strain was identified as *Aspergillus* sp. by comparing the ITS region of the rDNA sequence with that of the standard record (KJ938013). The ITS sequence has been submited to the GenBank (http://www.ncbi.nlm.nih.gov) with the accession number KP197676. The strain MEA11 was deposited at the Marine Culture Collection of China (MCCC 3A00599).

2. Previous Studies

The *Aspergillus* fungi, widely distributed in nature, were evidenced to be productive to produce metabolites bearing complicated structures or notable activities. In recent years, the chemistry of marine-derived fungi drew more and more attention from natural medicinal chemists. Strains belonging to the genera *Aspergillus* from marine resources were frequently isolated, and their metabolites were

* Corresponding authors: E-Mail: duxiaona_87@163.com
# These authors contributed equally to this work

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often studied, leading to the discovery of meroterpenoids [1], steroids [2], alkaloids [3-5], terpenoids [6-9], glucosides [10]. Some members showed remarkable bioactivities, such as varioxepine, which suppressed murine splenocyte proliferation activated by concanavalin A in vitro [4].

In our study, the HPLC fingerprint of the EtOAc extract of the strain *Aspergillus* sp. MEA11 displayed chromatographic peaks with similar ultraviolet spectra (λ_{max} 210, 245 nm), which suggested the presence of a series of analogs, and the extract (100 μg/mL) showed inhibition rate of 81% against the α-glucosidase. So we speculate that the strain may produce analogs with inhibitory effect on α-glucosidase. Subsequent chromatographic separations of the fermentation resulted in the identification of a new and seven known bisabolanes, which were evaluated for their inhibitory effects on α-glucosidase. Herein, the isolation and structural identification of these metabolites were described.

3. Present Study

4.

The fermentation was conducted in 30 erlenmeyer flasks (500 mL) with 75 g of rice and 90 mL of artificial sea-water, the contents were subsequent autoclaved. The flask was inoculated with spore inoculum and incubated for 30 days. The fermented materials were extracted with 4000 mL for three times to afford an EtOAc extract (2.4 g), which was chromatographed over ODS silica gel CC ($MeOH/H_2O$ = 20:80 to 100:0) to give ten fractions (Fr.A–Fr.J). Fr.F was further purified by ODS silica gel CC, eluting with $MeOH/H_2O$ (40:70→70:30), and followed by HPLC (37% $MeCN/H_2O$) to yield **6** (4.4 mg) and **1** (2.8 mg). Fr.G was separated by ODS using $MeOH/H_2O$ (30:70→100:0) as eluent to give seven subfractions (Fr.Ga–Fr.Gg). Fr.Ge was subjected to purification by HPLC using $MeCN/H_2O$ = 21:79 (3 mL/min) to yield **2** (17 mg) and **5** (t_R = 105 min, 46.4 mg). Fr.Gc was separated on a HPLC column with $MeCN/H_2O$ (20:80, 3 mL/min) as mobile phase to afford **4** (6 mg), **3** (5.5 mg), **7** (23 mg), and **8** (27.2 mg). Compound **8** was further purified by HPLC equipped with a chiral phase column ($MeOH/H_2O$, 90:10, 1 mL/min) to give **8a** (1.5 mg) and **8b** (1.7 mg)

11-Acetylated-12-hydroxysydonic acid (1): Colorless oil, $[\alpha]^{25}_D$ 0 (c = 0.1, CH_3OH); UV (MeOH) λ_{max} 219 (4.84), 245 (3.98) nm. ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS m/z: 363.1412 $[M + H]^+$ (calcd for $C_{17}H_{24}O_7Na^+$, 363.1414).

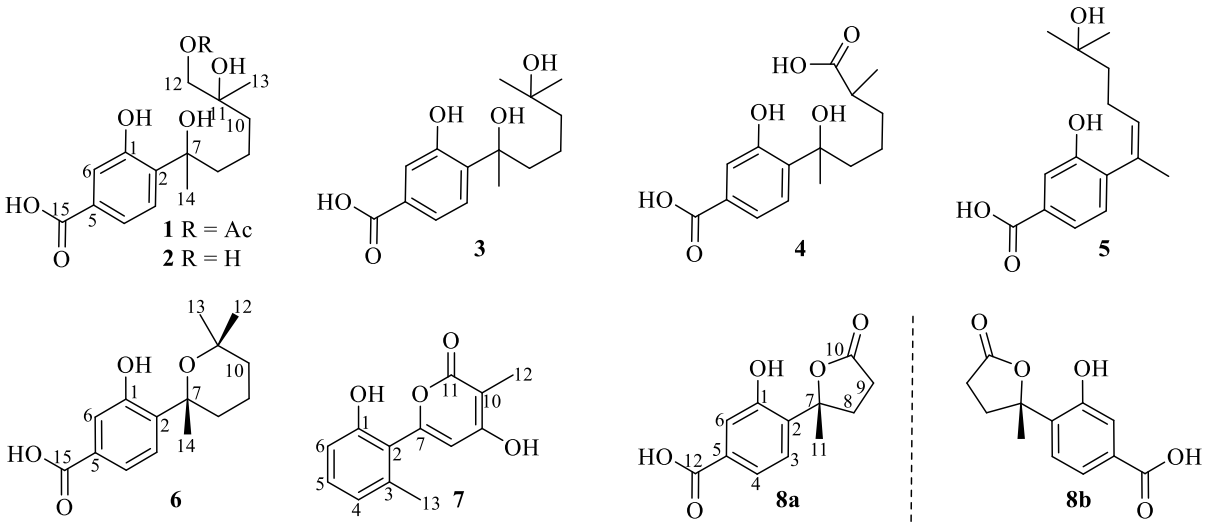

Figure 1. Compounds **1**–**8** from *Aspergillus* sp. MEA11

Compound **1**, a colorless oil, had the molecular formula $C_{17}H_{24}O_7$ as determined by the HRESIMS at m/z 363.1412 $[M + H]^+$ ($C_{17}H_{24}O_7$, calcd. 363.1414). The ^1H NMR spectrum displayed the resonances for three methyl singlets [δ_H 2.02 (3H, s), 1.61 (3H, s), 1.10 (3H, s)], three aromatic protons [δ_H 7.45

(1H, d, *J* = 8.2, 1.4 Hz), 7.37 (1H, d, *J* = 1.4 Hz, H-5), 7.28 (1H, d, *J* = 8.2 Hz) for a 1,2,4-trisubstituted benzene ring, a hydroxymethyl (δ_H 3.85), and several aliphatic protons. The ^{13}C NMR spectrum revealed the presence of 17 carbon resonances, which were classified by HSQC spectrum into two carbonyl carbons (δ_C 172.8, 170.1), three aromatic sp methine carbons (δ_C 127.8, 121.6, 118.6), three aromatic non-protonated carbons (δ_C 156.8, 137.9, 131.9), four sp^3 methylene carbons (δ_C 71.9, 43.8, 40.2, 19.2) including an oxygenated one (δ_C 71.9), two oxygenated non-protonated carbons (δ_C 77.7, 72.2), and three methyl carbons (δ_C 29.3, 23.9, 20.7). The above-mentioned information was very similar to those of 11,12-dihydroxysydonic acid (**2**) [11], indicating a phenolic bisabolane analog. The only structural difference between **1** and **2** was owing to the presence of an acetyl (δ_H 2.02; δ_C 20.7, 170.1) in **1**, suggesting **1** was acetylated derivative of **2**. The acetyl group was located at C-12 by the HMBC correlation from the hydroxymethyl protons at 3.85 to the carbonyl carbon of the acetyl group at 172.8.

The proposed structure of **1** was confirmed by detailed 2D NMR analyses (Figure 2). The HMBC correlations from the aromatic protons H-4 (δ_H 7.45) to C-2 (δ_C 137.9) and the carboxylic acid carbon at δ_C 170.1 (C-15), from H-6 (δ_H 7.37) to C-1 (δ_C 156.8), C-2, and C-15 (δ_C 170.1), and from H-3 (δ_H 7.28) to C-1 established a 3-hydroxy-benzoic acid moiety. The ^1H-^1H COSY relationship between H_2-9 (δ_H 1.28) and H_2-8 (δ_H 1.98, 1.81), H_2-10 (δ_H 1.44) assigned a spin system containing three methylenes, additional HMBC correlations from H_3-14 (δ_H 1.61) to C-2 (δ_C 137.9), C-7 (δ_C 77.7), C-8 (δ_C 43.8) and from H_2-12 (δ_H 3.85) and H_3-13 (δ_H 1.10) to C-10 (δ_C 40.2), C-11 (δ_C 72.2) established a side chain locating at C-2 (δ_C 137.9).

Table 1. NMR Data for **1** in methanol-d_4 (^1H NMR in 400MHz, ^{13}C NMR in 100 MHz)

position	**1**		**2**		**3**	**4**	**5**	**6**	**7**	**8**
	δ_C, type	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)	δ_C	δ_C	δ_C	δ_C	δ_C	δ_C
1	156.8, C		156.8, C		156.8	156.8	156.6	158.1	158.1	154.6
2	137.9, C		137.9, C		134.6	137.9	137.7	132.2	121.4	136.6
3	127.8, CH	7.28, d (8.2)	127.8, CH	7.28, d (8.1)	128.9	127.7	127.7	126.1	139.7	126.0
4	121.6, CH	7.45, dd (8.2, 1.4)	121.5, CH	7.44, dd (8.1, 1.5)	121.7	121.6	121.6	122.0	122.3	121.8
5	131.9, C		132.0, C		130.2	131.6	131.7	137.5	131.8	132.4
6	118.6, CH	7.37, d (1.4)	118.6, CH	7.36, d (8.1)	118.7	118.7	118.5	119.1	114.3	117.9
7	77.7, C		77.8, C		83.3	77.9	77.8	78.8	20.0	88.5
8	43.8, CH_2	1.98, m; 1.81, m	44.0, CH_2	2.0, m; 1.81, m	41.0	43.9	43.1	34.8	158.1	34.8
9	19.2, CH_2	1.28, m	19.2, CH_2	1.40, m; 1.29, m	19.1	19.9	22.8	17.6	105.9	29.6
10	40.2, CH_2	1.44, m	39.7, CH_2	1.40, m	39.6	44.9	35.1	37.7	167.9	179.5
11	72.2, C		73.7, C		73.6	71.5	40.6	76.2	99.8	26.4
12	71.9, CH_2	3.85, s	70.3, CH_2	3.29, s	70.3	29.2	181.1	32.3	169.6	169.5
13	23.9, CH_3	1.10, s	23.6, CH_3	1.06, s	22.7	29.1	17.6	25.2	8.4	
14	29.3, CH_3	1.61, s	28.8, CH_3	1.61, s	23.7	28.9	28.8	31.7		
15	170.1, C		170.1, C		168.0	169.9	170.2	169.7		
-OCH_3	20.7, C	2.02, s								
	172.8, C									

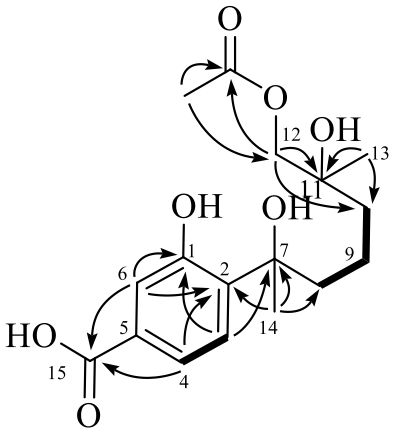

Figure 2. Key HMBC (⟶) and ^1H-^1H COSY (▬) correlations of **1**

In order to determine the absolute configuration of C-7 in **1**, the specific rotation and the ECD spectrum of **1** were measured. As a result, the specific rotation of **1** was close to zero and the cotton effect in the experimental ECD spectrum was negligible, indicating the racemic nature of **1**. Further chiral resolution of **1** on a chiral column failed. Compound **1** was named 12-acetoxy-11-hydroxysydonic acid according to the structure of 11,12-dihydroxysydonic acid (**2**).

The remaining compounds were identified to be 11,12-dihydroxysydonic acid (**2**) [11], hydroxysydonic acid (**3**) [11], aspergoterpenin B (**4**) [12], engyodontiumone J (**5**) [13], sydowic acid (**6**) [14], penicipyran A (**7**) [15], (+)-1-hydroxyboivinianic acid (**8a**) [16], (–)-1-hydroxyboivinianic acid (**8b**) [16] by comparisons of the NMR data (Table 1) with those reported in the literature. Compounds **4** and **7** were isolated from natural resources for the second time, and the NMR data of **7** recorded in methanol-d_4 were reported for the first time.

Compounds **1**–**8** were tested for their inhibitions on the α-glucosidase, compounds **6**, **7**, and **8** exhibited marked inhibitory effect with IC_{50} values of 176, 89, 232 μM, respectively, which were more active than that of the positive control acarbose (387 μM).

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

ORCID
Xin Liu: 0000-0002-9044-9308
Wenzhen Lin: 0000-0002-7517-6364
Ruzhen Liu: 0000-0003-2111-4237
Minghuang Ling: 0000-0002-0976-1856
Chiming Guo: 0000-0002-3614-6453
Hongyan Meng: 0000-0003-4122-3887
Ying Guo: 0000-0001-5408-1000
Wei Xu: 0000-0002-3265-7475
Xiaona Du: 0000-0002-6473-7613

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